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Shear inactivation of cellulase of *Trichoderma reesei*

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Inactivation of the cellulase of Trichoderma reesei *(EC 3.2.1.4) by shear, is of sufficient magnitude to merit consideration in the design of equipment for the enzymatic hydrolysis of cellulose. The inactivation constant,* k_d, *is a function of the flow rate of the enzyme solution through a fine capillary tube.* k_d *increased slowly at low shear stress, and much more rapidly when the shear stress was greater than 15 dynes* cm^{-2}.

Introduction

Recently in our laboratories, a significant deactivation of cellulase [1,4-(1,3; 1,4)-β-D-glucan 4-glucanohydrolase, EC 3.2.1.4], was observed during enzymatic hydrolysis of cellulose in shaking flasks.[1] Several factors, including temperature, pH and the presence of antibiotics, metal ions, surfactants and other chemicals, that affect stability of enzymes have been studied and the results reported.[1–6]

In this paper, the effect of shear on the stability of cellulase is reported. Other investigators have studied shear inactivation of catalase, rennin, carboxypeptidase[7,8] and urease.[9,10] It is anticipated that the results reported here for cellulase could be useful in the design and control of the cellulose hydrolysis process, where agitation must be provided to suspend the solid substrate, and where cellulase deactivation due to shear is significant.

Materials and methods

A crude cellulase preparation from the Rutgers University's mutant of *Trichoderma reesei* C-30[11] was diluted to a protein concentration of 1.25 mg ml^{-1} buffer (0.025 M citrate, pH 5.0). Tetracycline (0.002 mg ml^{-1}) was added as a preservative.[1] The enzyme solution (200 ml) was placed in the reservoir; 29 ml of this flowed into the line. The enzyme solution was recirculated under varying conditions (*Table 1*) using a peristaltic pump (Cole Parmer pump head 7018). Samples were tested for cellulase action, against crystalline cellulose (Avicel 102) by measuring the sugar produced from a 1.25% suspension over 60 min at 50°C.[1]

Results and discussion

The residual cellulase activity was measured as a function of time during which the cellulase was subjected to the shear, and the results are shown in *Figure 2*. During the first 48 h period the deactivation followed first order kinetics reasonably well showing linear correlation on a semilogarithmic plot. The deactivation constants, k_d, under varying conditions of shear rates were determined from *Figure 2*. The viscosity, μ, of the enzyme solution used in this experiment was determined at 1.54 centipoise (c.p.), and the Reynolds number, N_{Re}, under the conditions of maximum shear stress, was estimated to be 730, indicating that the flow of

Table 1 Effect of flow rate on deactivation of *T. reesei* cellulase

Curves in *Figure 2*	Flow rate, Q (ml min^{-1})	Pressure drop, ΔP (cm Hg)	10^{-4} × Shear rate, γ (min^{-1})	Shear stess, τ (dyne cm^{-2})	10^{-2} × Deactivation constant, k_d (h^{-1})
F	36	13.3	3.1	10.7	0.67
C	58.5	18.8	5.0	15.2	0.78
H	78.5	24.6	6.7	19.8	1.02
M	106	28.5	9.0	22.9	2.27

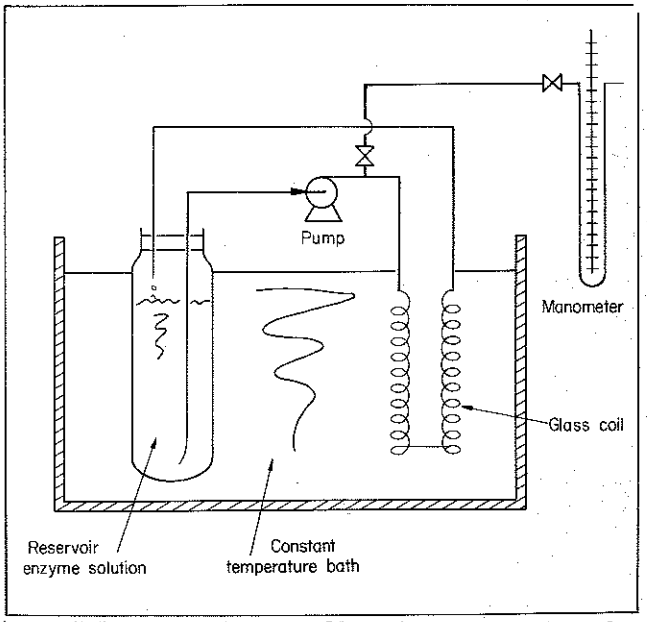

Figure 1 Experimental set-up used for evaluation of the effect of shear on cellulase deactivation. The cellulase reservoir, glass coil with silicone tubing connections (0.2 cm int. diam. and 550 cm total length) were immersed in a 50°C constant temperature bath

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enzyme solution in the thin tubing was within the laminar regime (see below for estimation).

Using the Hagan–Poiseuille equation to calculate the values in *Table 1*.

$$\text{Viscosity,} \quad \mu = \frac{(\Delta P)\, gc\, \pi R^4}{8LQ}$$

$$= 1.54 \text{ c.p.}$$

Reynolds number, N_{Re}:

$$N_{Re} = \frac{\rho v D}{\mu} = 730$$

Shear stress, τ:

$$\tau \text{ (mean)} = \int_0^R \tau_r \frac{2\pi r\, dr}{\pi R^2}$$

$$= \frac{(\Delta P) R}{3L}$$

Shear rate, γ:

$$\dot{\gamma} \text{ (mean)} = \frac{\tau \text{ (mean)}}{\mu} = \frac{8Q}{3\pi R^3}$$

assuming that $n = 1$ in $\tau = \mu(\dot{\gamma})^n$.

To determine the effect of shear on the enzyme activity, the deactivation constant, k_d, was plotted against the shear stress and shear rate (*Figure 3*). The deactivation constant increased with an increase in shear stress. In particular, when the shear stress was greater than ~15 dynes cm^{-2}, the deactivation constant increased sharply, indicating that severe deactivation of cellulase occurred.

The conditions that we have studied (50°C, pH 5.0, high protein, long time) are those under which the enzyme is used in the cellulose hydrolysis process. They differ from the less severe conditions (4°C, optimum pH, low protein, shorter times) used in previous studies.[7–10] As we have shown,[1] the effect is primarily on the *exo*-glucanase cellobiohydrolase (CBH) component, not on the *endo*-glucanase component, of cellulase. As a result the inactivation effect is most apparent in digestion, where the cellulose has a high degree of crystallinity (e.g. Avicel; cotton) and least apparent on celluloses of low crystallinity (Walseth, ball-milled cellulose, CMC). Elimination of the shear effect would result in a longer life for the CBH, with a consequent increase in the extent of cellulose digestion and the possibility of CBH recovery from the digests.

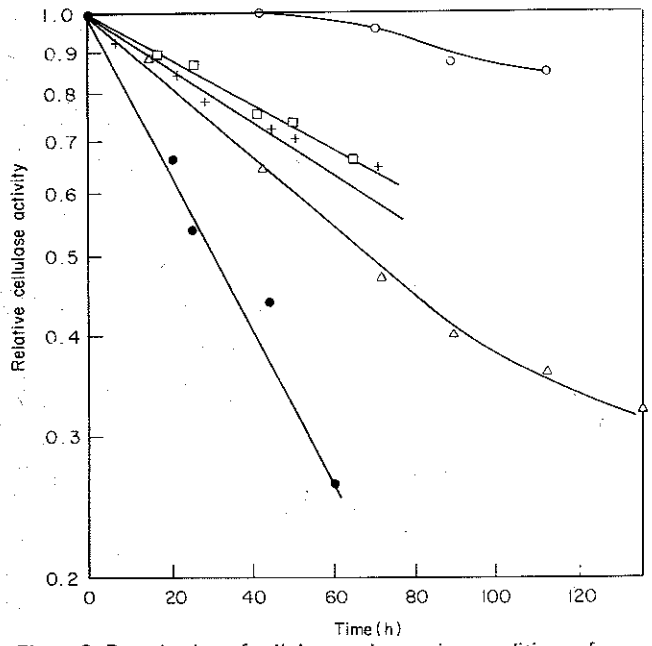

Figure 2 Deactivation of cellulase under varying conditions of shear (see *Table 1*). The residual cellulase activity is plotted against time of operation during which the cellulase solution was subjected to shear. ○, Control; □, F; +, C; △, H; ●, M. The slope represents deactivation constant (k_d)

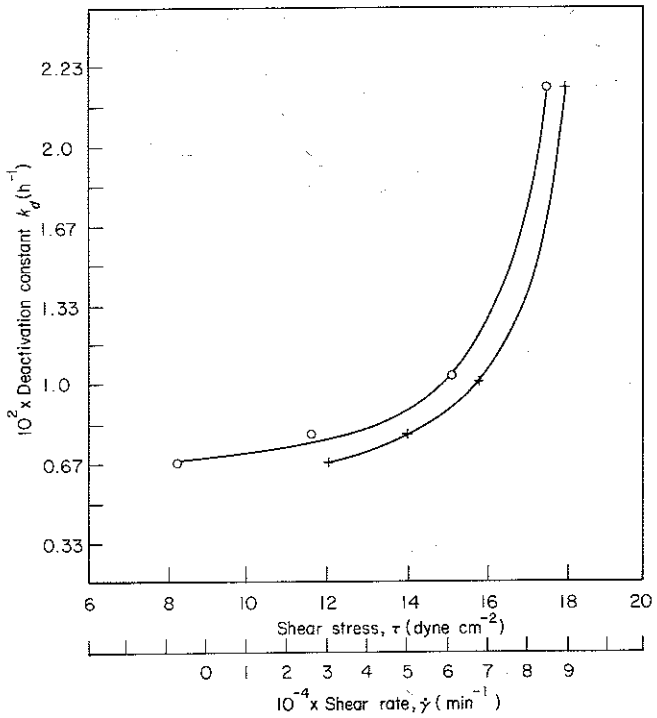

Figure 3 The effect of shear rate, $\dot{\gamma}$ (+), and shear stress, τ (○), on the deactivation constant, k_d, of cellulase

The results suggest that the deactivating effect of shear may be one of the most important factors responsible for slowing down the reaction rate during the enzymatic hydrolysis of cellulose. Until now, this reduction in rate has been attributed to product inhibition, adsorption of enzyme on lignocellulosics, temperature inactivation and decreased susceptibility of the residual cellulose.[1–6] The shear effect is of such a magnitude that it, too, must be taken into consideration for an improved process design for cellulose hydrolysis.

Methods of preventing the shear effect are being investigated.

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